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Application Note

Quantitation of Over the Counter Cold Medicine Formulations using UPLC Technology

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Abstract

In this application note a fast, high-resolution UPLC chromatographic method was developed for pharmaceutical formulations targeted to relieve symptoms of the common cold by utilizing a new High Strength Silica (HSS) UPLC stationary phase.

Introduction

Pharmaceutical formulations used to treat the common cold often contain multiple active ingredients to treat different symptoms. These actives can include combinations of decongestants, antihistamines, pain relievers, cough suppressants, and expectorants in addition to numerous excipients, all of which exhibit different chemical properties, including polarity. It is this range of analyte polarity that often makes chromatographic methods development difficult.

In this study, we used Waters UltraPerformance LC (UPLC) technology to develop a single chromatographic method for the analysis of the most common pharmaceutical formulations targeted to relieve symptoms associated with the common cold. Utilizing UPLC for this analysis improves productivity by enabling the separation of such complex mixtures while maintaining a high level of chromatographic resolution in a rapid analysis time.

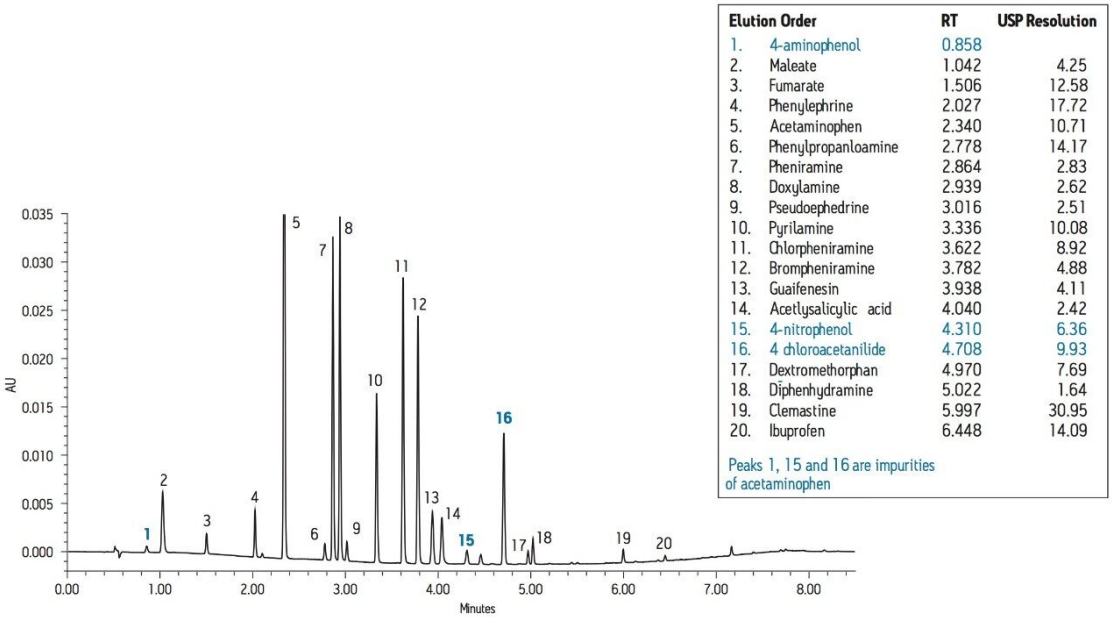
We used a new High Strength Silica (HSS) UPLC stationary phase to develop a single chromatographic method for the analysis of a number of possible formulation compositions. This stationary phase was selected due to its ability to enhance the retention of polar analytes while also having good chromatographic selectivity of hydrophobic species.

This methodology was then applied to several over-the-counter (OTC) formulations to quantitate the accuracy of reported concentrations of the active ingredients.

Experimental

A mixture of standards of 20 common components of cold medicine formulations including active

ingredients, impurities and counter ions was separated on a 2.1 x 100 mm, ACQUITY UPLC HSS T3, 1.8 µm Column, as depicted in Figure 1.



Elution Order		RT	USP Resolution
1.	4-aminophenol	0.858	
2.	Maleate	1.042	4.25
3.	Fumarate	1.506	12.58
4.	Phenylephrine	2.027	17.72
5.	Acetaminophen	2.340	10.71
6.	Phenylpropanloamine	2.778	14.17
7.	Pheniramine	2.864	2.83
8.	Doxylamine	2.939	2.62
9.	Pseudoephedrine	3.016	2.51
10.	Pyrilamine	3.336	10.08
11.	Chlorpheniramine	3.622	8.92
12.	Brompheniramine	3.782	4.88
13.	Guaifenesin	3.938	4.11
14.	Acetylsalicylic acid	4.040	2.42
15.	4-nitrophenol	4.310	6.36
16.	4 chloroacetanilide	4.708	9.93
17.	Dextromethorphan	4.970	7.69
18.	Diphenhydramine	5.022	1.64
19.	Clemastine	5.997	30.95
20.	Ibuprofen	6.448	14.09

Peaks 1, 15 and 16 are impurities of acetaminophen

Figure 1. UPLC separation of 20 common cold medicine active ingredients, impurities and counter ions.

This methodology was then applied to two OTC liquid formulations. A four-point calibration curve from 1 to 1,000 µg/mL was used to quantitate the accuracy of the reported values of the active ingredients respective to each OTC formulation.

Sample Preparation

Reference standards for the mixture depicted in Figure 1 were prepared in a solution of 75:25 (v/v) water:methanol containing 0.2% formic acid at a concentration of 25 µg/mL for each component except acetaminophen, which was 12.5 µg/mL and its respective impurities, 4-aminophenol, 4-nitrophenol and 4-chloroacetanilide, were prepared at a concentration of 2.5 µg/mL.

Individual stock solutions of the active ingredients of OTC formulation 1 (pseudoephedrine and diphenhydramine) were prepared at 2,000 µg/mL in a solution of 75:25 (v/v) water:methanol containing 0.2% formic acid. A mixture of the two actives was prepared at 1,000 µg/mL followed by serial dilutions of each subsequent concentration to make calibration standards at the concentrations of 100, 10 and 1 µg/mL.

A similar process was followed for the stock solution preparation of the active ingredients of OTC formulation

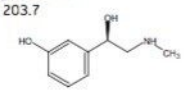
2 (acetaminophen, doxylamine and dextromethorphan). Individual stock solutions were prepared at a concentration of 3,000 µg/mL in a solution of 75:25 (v/v) water:methanol containing 0.2% formic acid. A mixture of the three actives was prepared at 1,000 µg/mL followed by serial dilutions of each subsequent concentration to make calibration standards at the concentrations of 100, 10 and 1 µg/mL.

The OTC liquid formulations were obtained from a local pharmacy and diluted with a 75:25 (v/v) methanol:water solution and directly injected on column without any further sample treatment or filtration. OTC formulation 1 was diluted 1:9 for the quantitation of pseudoephedrine and diphenhydramine. OTC formulation 2 was diluted 1:39 for the quantitation of doxylamine and dextromethorphan and diluted 1:199 for the quantitation of acetaminophen.

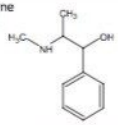
Chemical structures and classifications

Decongestants

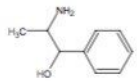
phenylephrine HCl
m.w. 203.7



pseudoephedrine
m.w. 165.23

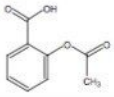


phenylpropanolamine HCl
m.w. 187.7

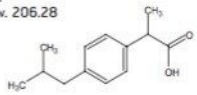


Pain relievers

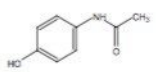
acetylsalicylic acid
m.w. 180.16



ibuprofen
m.w. 206.28



acetaminophen
m.w. 151.16

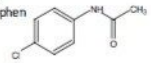


Impurities

4-aminophenol
m.w. 109.13
Impurity
of acetaminophen

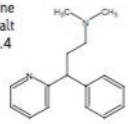
4-nitrophenol
m.w. 139.11
Impurity of
acetaminophen

4-chloroacetanilide
m.w. 169.61
Impurity of
acetaminophen

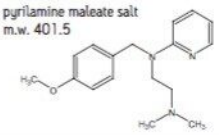


Antihistamines

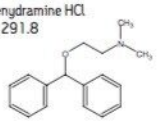
pheniramine
maleate salt
m.w. 356.4



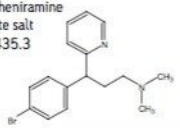
pyrilamine maleate salt
m.w. 401.5



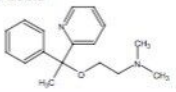
diphenydramine HCl
m.w. 291.8



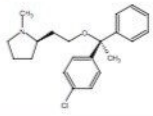
brompheniramine
maleate salt
m.w. 435.3



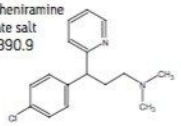
doxylamine succinate salt
m.w. 388.5



clemastine
fumarate
salt
m.w.
460.2

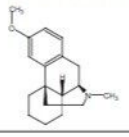


chlorpheniramine
maleate salt
m.w. 390.9



Cough suppressant

dextromethorphan HBrmonohydrate
m.w. 370.3

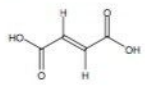


Expectorant

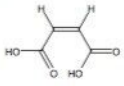
guaifenesin glyceryl ether
m.w. 198.22

Counter ions

fumarate
m.w. 116.07



maleate
m.w. 116.07



LC Conditions

LC system:	Waters ACQUITY UPLC System with Column

	Heater Module and ACQUITY UPLC Tunable UV (TUV) Detector
Software:	Empower 2, build 2154
Column:	ACQUITY UPLC HSS T3 2.1 x 100 mm, 1.8 µm Part Number: 186003539
Mobile phase:	A: 0.15% CF_3COOH in H_2O B: 0.02% CF_3COOH in 75:25 (v/v) ACN:MeOH
Flow rate:	0.6 mL/min
Injection:	1.0 µL
Loop size:	2.0 µL
Inject mode:	Partial loop with needle overfill
Column temp.:	30 °C
Detection:	UV @ 254 nm
Sampling rate:	20 Hz
Time constant:	0.2

Gradient

Time	%A	%B	Curve
0.0	99.9	0.1	
0.5	99.9	0.1	6

Time	%A	%B	Curve
1.7	87.0	13.0	6
3.6	67.0	33.0	6
7.5	1.0	99.0	6
8.0	1.0	99.0	6
8.5	99.9	0.1	6
9.5	99.9	0.1	6

Results and Discussion

Linearity

Serial dilutions from stock solutions were made to obtain four concentrations per calibration curve ranging from 1 to 1,000 µg/mL. Each concentration was injected in triplicate. A linear regression was used for doxylamine and dextromethorphan. A linear regression with weighting (1/X) was used for acetaminophen, pseudoephedrine and diphenhydramine. Table 1 lists the correlation coefficient and line equation for each analyte. Accepted value is greater than 0.995 for all sample components.

Component	R^2	Line Equation
acetaminophen	0.9997	Y = 7.41e006 X +1.70e003
dextromethorphan HBr	1.0000	Y = 8.59e004 X + 5.58e001
diphenhydramine HCl	0.9998	Y = 1.27e005 X - 9.70e-001
doxylamine succinate	0.9999	Y = 2.12e006 X + 2.49e002
pseudoephedrine HCl	0.9998	Y = 9.44e004 X + 4.22e001

Table 1. Linearity results.

Quantitation of OTC Formulation 1

OTC formulation 1 was diluted 1:9 with a 75:25 methanol:water solution and directly injected on column. As depicted in Figure 2, the active ingredients, pseudoephedrine and diphenhydramine, are well resolved from the excipients. As listed in Table 2, when quantitated against the calibration curve, percent recoveries for pseudoephedrine and diphenhydramine were 82% and 102%, respectively.

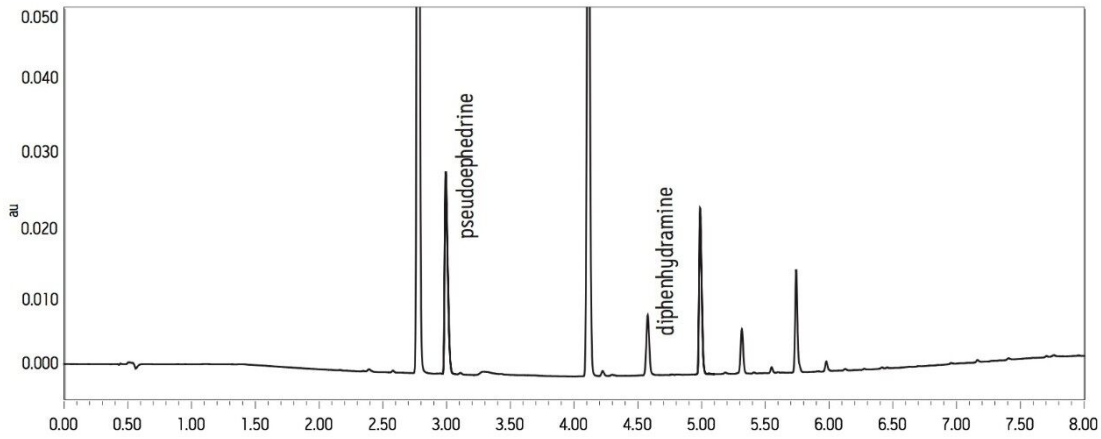


Figure 2. UPLC separation of OTC formulation 1 diluted 1:9.

Component	Reported Concentration mg/mL	Calculated Concentration mg/mL	% Recovery	Amount on Column mg/mL	Dilution Factor
acetaminophen	33.333	26.6	79%	0.133	200
doxylamine succinate	0.416	0.32	77%	0.008	40
dextromethorphan HBr	1	0.8	80%	0.02	40

Table 2. Percent recoveries of active ingredients in OTC formulation 1.

Quantitation of OTC Formulation 2

OTC formulation 2 was diluted 1:39 with a 75:25 methanol:water solution for the quantitation of doxylamine and dextromethorphan and diluted 1:199 for the quantitation of acetaminophen. These solutions were directly injected on column. As depicted in Figures 3 and 4, active ingredients acetaminophen, doxylamine and dextromethorphan, are well resolved from the excipients. As listed in Table 3, when quantitated against the calibration curve, percent recoveries for acetaminophen, doxylamine and dextromethorphan were 79%, 77% and 80%, respectively.

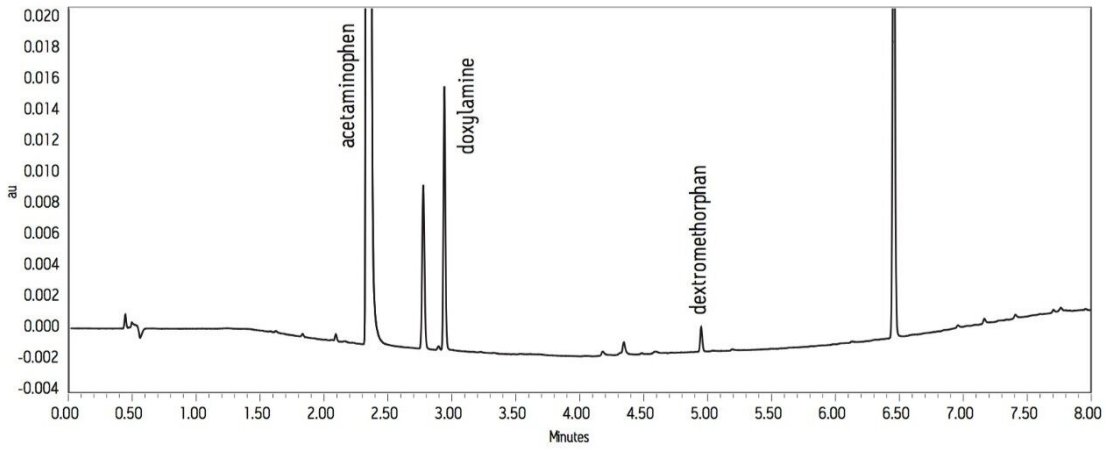


Figure 3. UPLC separation of OTC formulation 2 diluted 1:39 for quantitation of doxylamine and dextromethorphan.

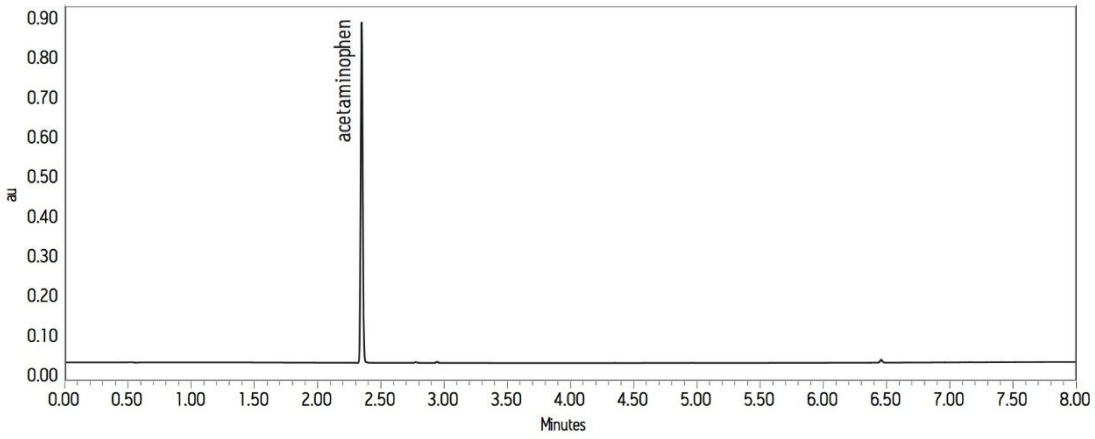


Figure 4. UPLC separation of OTC formulation 2 diluted 1:199 for quantitation of acetaminophen.

Component	Reported Concentration mg/mL	Calculated Concentration mg/mL	% Recovery	Amount on Column mg/mL	Dilution Factor
acetaminophen	33.333	26.6	79%	0.133	200
doxylamine succinate	0.416	0.32	77%	0.008	40
dextromethorphan HBr	1	0.8	80%	0.02	40

Table 3. Percent recoveries of active ingredients in OTC formulation 2.

Conclusion

A fast, high-resolution UPLC chromatographic method was developed for pharmaceutical formulations targeted to relieve symptoms of the common cold by utilizing a new High Strength Silica (HSS) UPLC stationary phase. This stationary phase was selected due to its ability to enhance the retention of polar analytes while also having good chromatographic selectivity of hydrophobic species. This single chromatographic method can be used to analyze a number of possible formulation compositions containing different active ingredients.

By utilizing UPLC technology, improvements in productivity were realized by enabling the separation of complex mixtures while maintaining a high level of chromatographic resolution in a rapid analysis time. This

results in a higher degree of confidence in results due to the ease of analyst familiarization with a single methodology, reducing the need for repeat analysis.

When we applied this methodology to over-the-counter (OTC) formulations, adequate resolution for the active ingredients from the excipients was observed. Analyte recoveries ranged from 77 to 102% of their reported value.

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